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PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
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אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
(Name and address of applicant, and in case of body corporate-place of incorporation)

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Inventors: Paul Gregor, Nicholas Harris, Juraj Koppel

Owner, by virtue of The Law and Assignment — בעל אמצאה מכח הדין והעברה

of an invention the title of which is: — ששמה הוא:

(בעברית) (Hebrew): תכשירים רפואיים המכילים ניגזרות THIENO [2,3-C]PYRIDINE והשימוש בהם

(באנגלית) (English): PHARMACEUTICAL COMPOSITIONS COMPRISING THIENO [2,3-C]PYRIDINE DERIVATIVES AND USE THEREOF

hereby apply for a patent to be granted to me in respect thereof. — מבקש בזאת כי ינתן לי עליה פטנט

*בקשת חלוקה - Application of Division	*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
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Ref.: RMX/002 IL

היום 5 בחודש פברואר שנת 2003
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תכשירים רפואיים המכילים ניגזרות **THIENO [2,3-C]PYRIDINE** והשימוש בהם

PHARMACEUTICAL COMPOSITIONS COMPRISING THIENO [2,3-C]PYRIDINE DERIVATIVES AND USE THEREOF

RMX/002 IL

PHARMACEUTICAL COMPOSITIONS COMPRISING THIENO [2,3-C]PYRIDINE DERIVATIVES AND USE THEREOF

FIELD OF INVENTION

The present invention relates to pharmaceutical composition comprising thieno[2,3-c]pyridine compounds capable of inhibiting the interactions between effector cell adhesion molecules (ECAMs), particularly selectins, and glycosaminoglycans (GAGs), particularly heparan sulfate glycosaminoglycans (HS-GAGs), and to methods for the treatment or prevention of diseases or disorders related to cell adhesion and cell migration, particularly for the treatment or prevention of inflammatory and autoimmune diseases and disorders, as well as of viral diseases and amyloid disorders.

BACKGROUND OF THE INVENTION

Inflammatory response is mediated primarily by white blood cells, the neutrophils. Neutrophils circulate in the blood where they reversibly interact with the vascular endothelium. In response to inflammatory stimuli, neutrophils adhere tightly to the vascular endothelium, migrate (extravasate) through the vessel wall, and subsequently move along a chemotactic gradient toward the inflammatory stimulus. The interaction of neutrophils with vascular endothelial cells is thus an essential initial step in the acute inflammatory response. Selectins play a key role in the inflammatory cascade of events, as they are responsible for the initial attachment of blood borne neutrophils to the vasculature. Preventing selectin-mediated cell adhesion can ameliorate or circumvent the deleterious consequences of inflammation. Therefore, selectins are the prime target for the therapy of cell-adhesion disorders, specifically for treatment of inflammation.

Selectins regulate neutrophil and lymphocyte adhesion to and entry into lymphoid tissues and sites of inflammation (Rosen, 1990 Am. J. Respir. Cell. Mol. Biol., 3:397-402). The three known selectins are E-selectin (formerly known as ELAM.1), P-selectin (formerly known as PADGEM, GMP-140, or CD61) and L-selectin (formerly known as mLHR, Leu8, TQ-1, gp90, MEL, Lam-1, or Lecam-1) (Lasky, Annu. Rev. Biochem. 64:113, 1995; Kansas Blood 88:3259, 1996). Each selectin is regulated differently, and participates in a different manner in the process of inflammation or immunity. The lectin domains of each selectin are critical to the

adhesive functions of the proteins. The selectins capture leukocytes in the blood stream and mediate their intermittent attachment to specific sites, with consequent leukocyte "rolling" along the endothelial cell surface. This capture allows the cascade of secondary, tighter cell-adhesive events to take place. Both P-selectin and E-selectin are inducible adhesion proteins for neutrophils and monocytes (Johnston et al., 1989 Cell 56:1033-1044). In contrast to these vascular selectins, L-selectin is constitutively expressed by leukocytes and mediates lymphocyte adhesion to peripheral lymph node high endothelial venules, and neutrophil adhesion to cytokine-activated endothelial cells (Spertini et al., 1991 J. Immunol. 147:2565-2573). In pathological conditions involving the immune system, it may be L-selectin that plays the most significant role (Shimizu et al., Immunol. Today 13:106, 1992; Picker et al., Annu. Rev. Immunol. 10:561, 1992).

Buerke et al. demonstrated the important role of selectins in inflammatory states such as ischemia-reperfusion injury in cats (Buerke, M. et al., J. Clin. Invest. (1994) 93:1140). Turunen et al. demonstrated the role of sLex and L-selectin in site-specific lymphocyte extravasation in renal transplants during acute rejection (Turunen, J. P. et al., Eur. J. Immunol. (1994) 24:1130). P-selectin was shown to be centrally involved in acute lung injury. Mulligan et al. reported strong protective effects using anti-P-selectin antibody in a rodent lung injury model. (Mulligan, M. S. et al., J. Clin. Invest., (1991) 90:1600, Mulligan, M. S. et al., Nature (1993) 364:149). A central role of P-selectin in inflammation and thrombosis was demonstrated by Palabrica et al. (Palabrica, T. et al., Nature (1992) 359:843). Recent publications on selectin ligands describe the use of L-selectin as an indicator of neutrophil activation (U.S. Patent No. 5,316,913 to Butcher et al.), and in assays for the inhibition of leukocyte *adhesion* (U.S. Patent No. 5,318,890 to Rosen et al.). The presence of L-selectin and E- or P-selectin ligands on mononuclear cells has implicated these receptor-ligand interactions in chronic inflammation. (L. Lasky Annu. Rev. Biochem. 64:113-39 (1995); "Selectin Family of *Adhesion* Molecules" by Michael Forrest and James C. Paulson in Physiology and Pathophysiology of Leukocyte *Adhesion,* Ed. by D. Niel Grangier and Deert Schmid-Schonbein, Oxford University Press, New York, N.Y., 1995). Monoclonal antibodies to L-selectin prevent neutrophil emigration into inflamed skin (Lewinsohn et al., 1987 J. Immunol. 138:4313), neutrophil and monocyte emigration into inflamed ascites (Jutila et al., 1989 J. Immunol. 143:3318), and neutrophil emigration into inflamed peritoneum. Jasin et al. provide support for

the use of antibodies in inhibiting neutrophil accumulation in inflamed synovium (Jasin et al., 1990 Arthritis Rheum. 33:S34; Koch et al., 1991 Lab. Invest. 64:313). Monoclonal antibody EL-246, directed against both L-selectin and E-selectin, attenuated sepsis-induced lung injury (Ridings, PC et al., 1995, Arch Surg. 1199-1208). Monoclonal antibody SMART is an L-selectin blocking antibody in clinical trials for trauma associated with multiple organ failure (this condition is believed to be due in part to infiltration of inflammatory cells). The anti-L-selectin antibody is expected to provide its therapeutic effect by preventing neutrophil adhesion to endothelium and it is active in vivo in a primate model of severe trauma (Critical Care Medicine 1999, 27, 1900-1907). It is believed that this monoclonal antibody will be also useful in the treatment of adult respiratory distress syndrome and myocardial infarction (Protein Design Labs, Company Press Release, 1999).

Glycosaminoglycans (also referred to herein and in the art as "GAG" or "GAGs") are naturally-occurring carbohydrate-based molecules implicated in the regulation of a number of cellular processes, including blood coagulation, angiogenesis, tumor growth and smooth muscle cell proliferation, most likely by interaction with effector molecules (Jackson et al. (1991) Physiological Reviews 71:481-539 and Kjellen et al. (1991) Ann. Rev. Biochem. 60:443-475). GAGs are often, but not always, found covalently bound to protein cores in structures called proteoglycans. Proteoglycan structures are abundant on cell surfaces and are associated with the extracellular matrix around cells. Heparan sulfate glycosaminoglycans (also referred to herein and in the art as "HS-GAGs") consist of repeating disaccharide units (the sugars are D-glucuronic acid and N-acetyl- or N-sulfo-D-glucosamine). The high molecular diversity of HS-GAGs is due to its unique sulfation pattern (Sasisekharan, R. and Venkataraman, G., Current Opinion in Chem. Biol., 2000, 4, 626-631; Lindahl, U. et al., 1998, J. Biol. Chem., 273, 24979-24982; Esko, J. and Selleck, S.B., 2002, Annu. Rev. Biochem., 71, 435-471). One of the most thoroughly studied HS-GAGs is the widely used anticoagulant heparin. Heparin is a highly sulfated form of heparan sulfate found in mast cells. Many important regulatory proteins bind tightly to heparin, including cytokines, growth factors, enzymes and cell adhesion molecules. Although interactions of proteins with GAGs such as heparin and heparan sulfate, are of great biological importance, the structural requirements for protein-GAG binding have not been well characterized. Ionic interactions are important in promoting protein-GAG binding and the spacing of the

charged residues may determine protein-GAG affinity and specificity. The HS-GAG paradigm provides new approaches and strategies for therapeutic intervention at the cell–tissue–organ interface. For example, identification of specific HS-GAG sequences that effect particular biological processes will enable the development of novel molecular therapeutics based on polysaccharide sequence. Synthetic HS-GAGs, or molecular mimics of HS-GAG sequences, may provide new approaches for combating health problems such as bacterial and viral infections, atherosclerosis, cancer, and Alzheimer's disease.

Selectins mediate their adhesive functions via lectin domains that bind to carbohydrate ligands. Emerging evidence indicates that glycosaminoglycans (GAGs), and in particular heparan sulfate glycosaminoglycans (HS-GAGs), are carbohydrate receptors with which the selectins interact (Nelson RM, et al., 1993, Blood 82, 3253-3258; Ma, YQ and Geng, JG, 2000, J. Immunol. 165, 558-565; Kawashima H., et al., 2000, J. Biol. Chem., Aug 18 issue; Giuffre, L. et al., 1997, J. Cell. Biol. 136, 945-956; Watanabe N., et al., 1999, J. Biochem. 125, 826-831; Li YF et al., 1999, FEBS Lett 444, 201-205). Consistent with this observation, heparin, HS-GAG and heparin-derived oligosaccharides block L-selectin-dependent adhesion directly (U.S. Patent No. 5,527,785 to Bevilacqua et al.). Furthermore, short sulfated heparin-derived tetrasaccharides reduced binding of neutrophils to COS cells expressing P-selectin (Nelson RM, et al., 1993, Blood 82, 3253-3258). The multivalent nature of HS may be an important factor in binding L-selectin under flow conditions (Sanders et al, ibid). The endothelial proteoglycans recognized by L-selectin are Heparan Sulfate Proteoglycans (HSPGs), rather than sialylated, fucosylated or sulfated glycoprotein ligands (Koenig, A., et al., 1998, J. Clin. Invest. 101, 877-889).

As the interactions between GAGs and selectins play an important role in cell-matrix and cell-cell adhesion, which are processes involved in certain diseases and disorders, modulating these interactions have therapeutic implication.

Bevilacqua et al (U.S. Patent No. 5,527,785) provide a method of modulating selectin binding in a subject by administering heparin-like oligosaccharides. The oligosaccharides act by binding to L- or P-selectin.

Xie X et al (JBC 275, 34818-25, 2000) described inhibition of L- and P-selectin mediated cell adhesion by sulfated saccharides, including carboxyl-reduced and sulfated heparin. While these molecules have been useful to show the utility of selectin blockers for treating inflammation, each has significant drawbacks as a

therapeutic, including short in vivo half-life, high cost, potential immunogenicity, and other possible side effects. A further limitation of these approaches is lack of efficient means to improve the pharmacological properties of these molecules.

International Patent Application No. WO 02/076173 discloses peptide derivatives that inhibit GAG molecules, specifically hyaluronic acid (HA).

US Patent No. 6,232,320 discloses the use of thieno[2,3-c]pyridines as inhibitors of cell adhesion useful as inhibitors of inflammation. The disclosed compounds are different from the compounds of the present invention as they posses a different heterocyclic system and do not posses sulfonylbenzoylamino group.

Japanese Patent Application JP 2001151779 discloses 4,5,6,7-Tetrahydrothieno[2,3-c]pyridines, pharmaceutical compositions, and TNF-α formation inhibitors containing them, also disclosed in Fujita M, et al. (Bioorg. Med. Chem. Lett. 2002.12, 1607-1611). The disclosed compounds are different from the compounds of the present invention since the former do not posses sulfonylbenzoylamino group.

Japanese Patent Application JP 2001151780 discloses novel 4,5,6,7-tetrahydrothieno[2,3-c]pyridines as inhibitors of TNF-alpha synthesis, also disclosed in Fujita et al. (Bioorg. Med. Chem. Lett. 2002, 12, 1897-1900). Again, the disclosed compounds are different from the compounds of the present invention since the former do not posses sulfonylbenzoylamino group.

SciFinder Scholar database, release 2002, lists 318 derivatives (as of December 9, 2002) of thieno[2,3-c]pyridine of general Formula I described herein below, but no utility or chemical synthesis data is described.

Chemical Diversity Labs Inc. (San Diego, CA), a supplier of chemical compounds, released a database named CombiLab Probe Libraries (June 2002 revision; 220,674 compound structures), which lists 438 derivatives of thieno[2,3-c] pyridine of general Formula I herein below, but no utility or chemical synthesis data is described.

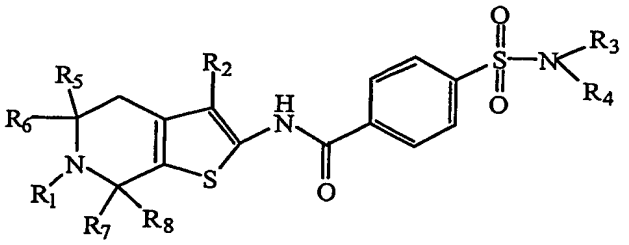
Nowhere in the background art is it taught or suggested that sulfonylbenzoylamino derivatives of thieno [2,3-c] pyridines have beneficial pharmaceutical activities.

SUMMARY OF THE INVENTION

It is an object of some aspects of the present invention to provide

pharmaceutical compositions comprising small organic compounds for medical and diagnostic use, wherein the small organic compounds are inhibitors of the interactions between effector cell adhesion molecules (ECAMs), specifically L-selectin and P-selectin with glycosaminoglycans (GAGs), specifically heparan sulfate glycosaminoglycans (HS-GAGs). Accordingly, these compositions are useful as inhibitors of cell-cell interactions mediated by L-selectin and P-selectin, particularly leukocyte adhesion, migration and infiltration. In addition, said compositions interact directly with HS-GAGs and are therefore useful as inhibitors of any HS-GAG mediated processes and conditions.

According to one aspect, the present invention provides pharmaceutical composition comprising as an active ingredient a compound of the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with alkyl groups; alkanoyl; arylcarbonyl optionally substituted at the aryl group; cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain C_1-C_6 alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of C_1-C_6 alkyl, alkoxy alkyl, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl, or R_3 and R_4 together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl

containing one or two heteroatoms;

R_5, R_6, R_7 and R_8 are selected from the group consisting of H or C_1-C_6 alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are C_1-C_6 alkyl, R_1 is hydrogen;

and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

According to one embodiment, R_1 is selected from the group consisting of methyl, ethyl, 1-methylethyl, phenylmethyl, acetyl, ethoxycarbonyl and R_5 =R_6 =R_7 =R_8 are hydrogen.

According to another embodiment, R_1 is hydrogen and R_5 =R_6 =R_7 =R_8 are hydrogens or methyl groups.

According to yet another embodiment, R_1 =R_5 =R_6 is methyl and R_7 =R_8 are hydrogens.

According to one embodiment R_2 is selected from the group consisting of cyano, methoxycarbonyl, ethoxycarbonyl, aminocarbonyl, methylaminocarbonyl, dimethylaminocarbonyl, pyrrolidinylcarbonyl, piperidinylcarbonyl, morpholinylcarbonyl, benzothiazol-2-yl.

According to one embodiment, R_3 and R_4 are selected from the group consisting of methyl, ethyl, propyl, butyl, methoxyethyl, chlorobutyl, cyanoethyl, phenyl, cyclopentyl, cyclohexyl, phenylmethyl, allyl or crotyl, R_3 and R_4 may be equal or different.

According to another embodiment, R_3 and R_4 form pyrrolidine, piperidine, 2-methyl, 3-methyl, 4-methyl or 3,5-dimethyl piperidine, perhydroazepine, morpholine, piperazine, 3,4-dihydro-1(2H)quinoline, 1,3,3-trimethyl-6-azabicyclo[3.2.1]oct-6-ane and their substituted derivatives such as piperazinyl-4-caboxylic acid ester, piperidinyl-4-carboxylic acid ester, piperidinyl-3-carboxylic acid ester.

According to certain preferred embodiment the present invention provides compositions comprising compounds of formula I selected from:

2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[(4-(3,4-dithydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl]benzoyl]amino]-4,5,6,7-

tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxamide ;

2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(morpholinylsulfonyl) benzoyl]amino]-3-(benzothiazol-2-yl)-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(diethylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester ;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine ;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine ;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo [3.2.1]oct-6-yl)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(methylphenylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-(morpholinylsulfonyl) benzoyl] amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine.

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-6-carboxylic acid ethyl ester;

2-[[4-[[4-(3-methyl-1-piperidinyl)]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-(phenylmethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(cyclohxylmethylamino}sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(di-2-propenylamino)sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxylic acid methyl ester;

2-[[4-[(di-2-methoxyethylamino)]sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-

tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo[3.2.1.]okt-6-yl)sulfonyl]benzoyl]amino] – 6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide

According to one embodiment the compositions of the present invention inhibit the binding of GAGs to GAG-specific-ECAMs.

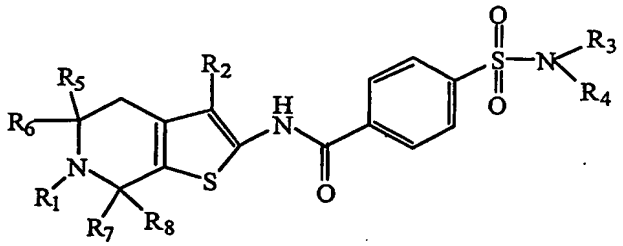
According to another embodiment the compositions of the present invention inhibit the interactions of HS-GAGs with selectins, specifically L-selectin and P-selectin.

According to yet another embodiment the compounds according to the compositions of the present invention bind directly to GAGs, specifically HS-GAG.

According to a further embodiment the compositions of the present invention inhibit leukocyte and neutrophil infiltration *in vivo*.

According to yet another embodiment the present invention provides a method for inhibiting cell adhesion and cell migration in vitro comprising the step of exposing the cells to at least one compound according to formula I in an amount sufficient to inhibit binding of GAGs to GAG specific ECAMs.

According to yet other aspects the present invention provides a method for the treatment or prevention of diseases and disorders related to cell adhesion and cell migration mediated by GAG-ECAM interactions, comprising the step of administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with alkyl groups; alkanoyl; arylcarbonyl optionally substituted at the aryl group;

cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain C_1-C_6 alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of C_1-C_6 alkyl, alkoxy alkyl, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl, or R_3 and R_4 together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms;

R_5, R_6, R_7 and R_8 are selected from the group consisting of H or C_1-C_6 alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are C_1-C_6 alkyl, R_1 is hydrogen;

and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

According to one embodiment the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders mediated by GAG-ECAM interactions wherein the GAGs are selected from the group consisting of heparan sulfate (HS-GAG), heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate, and derivatives and fragments thereof.

According to one currently preferred embodiment, the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders mediated by GAG-ECAM interactions wherein the GAG is HS-GAG.

According to yet another embodiment the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders mediated by GAG-ECAM interactions wherein the GAG specific ECAMs are selected from the group consisting of selectins, integrins fibronectin and cytokines.

According to one currently preferred embodiment, the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders mediated by GAG-ECAM interactions wherein the GAG specific ECAMs are selected from the group consisting of L-selectin and P-selectin.

According to one embodiment, the disease or disorder mediated by GAG-ECAM interactions may be an inflammatory process, an autoimmune process or

disease, platelet-mediated pathologies, tumor metastasis, viral diseases, coagulation disorders, atherosclerosis, amyloid disorders, and kidney disease.

According to another embodiment the inflammatory processes or disorders mediated by GAG-ECAM interactions are exemplified by, but not restricted to septic shock, post-ischemic leukocyte-mediated tissue damage, frost-bite injury or shock, acute leukocyte-mediated lung injury, acute pancreatitis, nephritis, asthma, traumatic shock, stroke, traumatic brain injury, nephritis, acute and chronic inflammation, including atopic dermatitis, psoriasis, uveitis, retinitis, and inflammatory bowel disease.

According to yet another embodiment the autoimmune diseases mediated by GAG-ECAM interactions are exemplified by, but not restricted to rheumatoid arthritis and multiple sclerosis.

According to yet further embodiment, the diseases or disorders mediated by GAG-ECAM interactions include those mediated by cell-cell, cell-virus, cell-matrix and cell-protein interactions, exemplified by, but not restricted to virus attachment to cell, cell adhesion, platelet aggregation, lymphocyte adhesion and migration, and amyloid fibril formation.

According to yet another embodiment, the pharmaceutical compositions according to the present invention are administered for the treatment or prevention of diseases and disorders associated with unwanted leukocyte or neutrophil infiltration.

According to one embodiment, the diseases and disorders associated with unwanted leukocyte or neutrophil infiltration are exemplified by, but not restricted to inflammatory processes such as post-ischemic leukocyte-mediated tissue damage, acute pancreatitis, septic shock, stroke, acute and chronic inflammation, Crohn's disease, rheumatoid arthritis, multiple sclerosis and leukemia.

According to yet further embodiment, the pharmaceutical compositions according to the present invention are administered for the treatment or prevention of diseases and disorders mediated by GAGs, specifically HS-GAG.

According to one embodiment, the diseases and disorders mediated by GAGs is selected from the group consisting of amyloid disorders: Alzheimer's disease and type II diabetes; viral diseases: hepatitis C and B, influenza, rhinovirus infections, cytomegalovirus infections, AIDS, respiratory syncytial virus infections; bacterial infections and malaria; kidney diseases; cancer and coagulation disorders.

Further embodiments and the full scope of applicability of the present invention

will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows heparin binding to L-selectin.

FIG. 2 demonstrates inhibition of L-selectin/IgG binding to immobilized heparin by soluble heparin.

FIG. 3 shows inhibition of L-selectin/IgG binding to heparin by anti-L-selectin antibody DREGG-55.

FIG. 4 Compound no. 5 inhibits neutrophil infiltration in mouse peritonitis in a dose dependent manner.

FIG. 5 demonstrates the anti-inflammatory properties of compound no. 5 in Paw Edema.

FIG. 6 shows inhibition of inflammation by compound no. 5 in the model of Delayed Type Hypersensitivity (DTH).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

The term "compound" refers to small organic molecule having a molecular weight less than 1500 Daltons and preferably between 300 to 1200 Daltons.

The term "HS-GAG" refers to heparan sulfate glycosaminoglycan. It includes fragments of heparan sulfate such as those that may be produced chemically, enzymatically or during purification. It includes the HS-GAG chains of proteoglycans such as heparan sulfate proteoglycans. HS-GAG may be free or attached to a linker, support, cell or protein, or otherwise chemically or enzymatically modified. HS-GAGs may be crude or purified from organs, tissues or cells.

The term "GAG" refers to glycosaminoglycans, including heparan sulfate (that is referred to in the art also as HS-GAG), heparin, chondroitin sulfate, dermatan sulfate and keratan sulfate. It includes the GAG chains of proteoglycans such as

heparan sulfate proteoglycan or chondroitin sulfate proteoglycan.

"HS-PG" refers to heparan sulfate proteoglycans.

"Heparin" is polysulfated polysaccharide, with no protein associated with it. As used herein, heparin refers to heparin prepared from different organs or species such as porcine intestinal mucosa heparin. It includes low molecular weight heparins, such as commercially available Fraxiparin, and other heparin derivatives, prepared or modified by chemical or enzymatic reaction.

"Heparin Derivatives" consist of products derived from heparin, made by one or more chemical or enzymatic modifications. The modifications are designed to change the activity of relevant groups of the molecules.

"Heparin Derived Oligosaccharides" are products made from heparin by controlled cleavage and subsequent purification.

"Heparan Derivatives" consist of products derived from heparan sulfate, made by one or more chemical or enzymatic modifications. The modifications are designed to change the activity of relevant groups of the molecules.

"Heparan Derived Oligosaccharides" are products made from heparan sulfate by controlled cleavage and subsequent purification.

The terms "L-selectin/IgG" and "P-selectin/IgG" refer to a selectin chimera molecule, in which an N-terminal portion of the selectin comprising the binding domain is fused to an IgG Fc region (Aruffo et al., Cell 67:35, 1991 and Foxall et al. J. Cell Biol. 117:895, 1992).

The term "GAG specific ECAM" means an effector cell adhesion molecule and refers to a carbohydrate-binding protein molecule involved in mediating cell adhesion, cell-cell and cell-matrix interaction and having a heparin-binding domain, such as L-selectin, P-selectin, integrins, fibronectin, and the like. It includes mutant proteins, protein domains, peptide fragments and the like, that retain the GAG binding domain.

The term "Inhibitor Compound" refers to a small organic molecule inhibiting the interaction (binding) between two molecules: (1) a GAG, exemplified by, but not restricted to heparin or HS-GAG and (2) a GAG specific ECAM, exemplified by, but not restricted to L-selectin, P-selectin or integrin.

The terms "inflammation", "inflammatory diseases", "inflammatory condition" or "inflammatory process" are meant as physiological or pathological conditions, which are accompanied by an inflammatory response. Such conditions include, but are not limited to sepsis, ischemia-reperfusion injury, Crohn's disease, arthritis,

multiple sclerosis, cardiomyopathic disease, colitis, infectious meningitis, encephalitis, acute respiratory distress syndrome, the various organ/tissue transplants (such as skin grafts, kidney, heart, lung, liver, bone marrow, cornea, pancreas, small bowel, organ/tissue rejection), an infection, a dermatitis, stroke, traumatic brain injury, inflammatory bowel disease and autoimmune diseases.

The term "treatment" or "treating" is intended to include the administration of the compound of the invention to a subject for purposes which may include prophylaxis, amelioration, prevention or cure of disorders mediated by cell adhesion or cell migration events, specifically selectin adhesion events, more specifically L-selectin and P-selectin-mediated adhesion events. Such treatment need not necessarily completely ameliorate the inflammatory response or other responses related to the specific disorder. Further, such treatment may be used in conjunction with other traditional treatments for reducing the disease or disorder condition known to those of skill in the art.

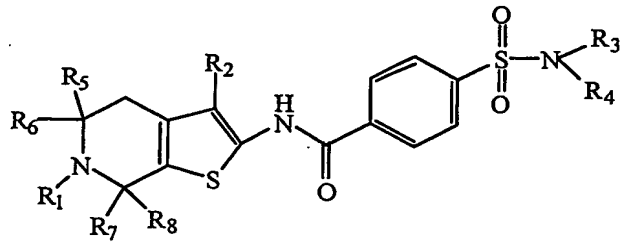
The methods of the invention may be provided as a "preventive" treatment before detection of, for example, an inflammatory state, so as to prevent the disorder from developing in patients at high risk for the same, such as, for example, transplant patients.

As used through this specification and the appended claims, the singular forms "a", "an" and "the" include the plural unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes mixtures of such compounds, reference to "a P-selectin", or "an L-selectin" includes reference to respective mixtures of such molecules, reference to "the formulation" or "the method" includes one or more formulations, methods and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

Pharmaceutical compositions according to the present invention

The present invention relates to pharmaceutical compositions comprising as an active ingredient at least one compound capable of inhibiting the interactions of glycosaminoglycans (GAGs), particularly heparan sulfate glycosaminoglycans (HS-GAG) with effector cell adhesion molecules (ECAMs), particularly GAG-specific ECAMs, specifically L-selectin and P-selectin.

According to some aspects the present invention provides pharmaceutical compositions comprising as an active ingredient a compound having the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with alkyl groups; alkanoyl; arylcarbonyl optionally substituted at the aryl group; cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain C_1-C_6 alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of C_1-C_6 alkyl, alkoxy alkyl, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl, or R_3 and R_4 together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms;

R_5, R_6, R_7 and R_8 are selected from the group consisting of H or C_1-C_6 alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are C_1-C_6 alkyl, R_1 is hydrogen;

and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

According to one embodiment, R_1 is selected from the group consisting of methyl, ethyl, 1-methylethyl, phenylmethyl, acetyl, ethoxycarbonyl and R_5 =R_6 =R_7 =R_8 are hydrogen.

According to another embodiment, R_1 is hydrogen and $R_5 = R_6 = R_7 = R_8$ are hydrogens or methyl groups.

According to yet another embodiment, $R_1 = R_5 = R_6$ is methyl and $R_7 = R_8$ are hydrogens.

According to one embodiment R_2 is selected from the group consisting of cyano, methoxycarbonyl, ethoxycarbonyl, aminocarbonyl, methylaminocarbonyl, dimethylaminocarbonyl, pyrrolidinylcarbonyl, piperidinylcarbonyl, morpholinylcarbonyl, benzothiazol-2-yl.

According to one embodiment, R_3 and R_4 are selected from the group consisting of methyl, ethyl, propyl, butyl, methoxyethyl, chlorobutyl, cyanoethyl, phenyl, cyclopentyl, cyclohexyl, phenylmethyl, allyl or crotyl, R_3 and R_4 may be equal or different.

According to another embodiment, R_3 and R_4 form pyrrolidine, piperidine, 2-methyl, 3-methyl, 4-methyl or 3,5-dimethyl piperidine, perhydroazepine, morpholine, piperazine, 3,4-dihydro-1(2H)quinoline, 1,3,3-trimethyl-6-azabicyclo[3.2.1]oct-6-ane and their substituted derivatives such as piperazinyl-4-caboxylic acid ester, piperidinyl-4-carboxylic acid ester, piperidinyl-3-carboxylic acid ester.

According to one embodiment, currently preferred compounds according to formula I are listed herein below:

2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[(4-(3,4-dithydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxamide ;

2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(morpholinylsulfonyl) benzoyl]amino]-3-(benzothiazol-2-yl)-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(diethylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester ;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-3-(benzothiazol-

2-yl)-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine ;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine ;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo [3.2.1]oct-6-yl)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(methylphenylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-(morpholinylsulfonyl) benzoyl] amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine.

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-6-carboxylic acid ethyl ester;

2-[[4-[[4-(3-methyl-1-piperidinyl)]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-(phenylmethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(cyclohxylmethylamino}sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(di-2-propenylamino)sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxylic acid methyl ester;

2-[[4-[(di-2-methoxyethylamino)]sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo[3.2.1.]okt-6-yl)sulfonyl]benzoyl]amino] –6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide.

Unless otherwise indicated, all chiral, diastereomeric and racemic forms of the compounds described in the present invention are also included in the present invention; the compounds may also have asymmetric centers. Many geometric isomers of olefins, C- and N- double bonds and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the

present invention. It will be appreciated that compounds of the present invention that contain asymmetrically substituted carbon atoms may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated.

When a bond to a substituent is shown to cross the bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring. When a bond joining a substituent to another group is not specifically shown or the atom in such other group to which the bond joins is not specifically shown, then such substituent may form a bond with any atom on such other group.

Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds. By stable compound or stable structure it is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

The term "substituted", as used herein, means that any one or more hydrogen on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

A "lead compound" is a compound in a selected combinatorial library, for which the assay has revealed significant effect relevant to a desired cell activity to be modulated. In the present case the property is the modulation of at least one biological activity associated with a GAGs or GAG-ECAM intercations.

The term "alkyl" refers to a straight or branched chain or cyclic hydrocarbon having 1-12 carbon atoms. In one embodiment, the alkyl has 1-10 carbons. In another embodiment, the alkyl has 1-8 carbons. In another embodiment, the alkyl has 1-6 carbons. In another embodiment, the alkyl has 1-4 carbons. The alkyl may be unsubstituted or substituted by one or more substituents, i.e. substituents which do not interfere with the biological activity of the compounds. Non-limiting examples of suitable substituents include but are not limited to halo, hydroxy, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_1-C_{10} alkoxy, C_7-C_{12} aralkyl, C_7-C_{12} alkaryl, C_1-C_{10} alkylthio, arylthio, aryloxy, arylamino, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, di(C_1-C_{10})-

alkylamino, C_2-C_{12} alkoxyalkyl, C_1-C_6 alkylsulfinyl, C_1-C_{10} alkylsulfonyl, arylsulfonyl,aryl, hydroxy, hydroxy(C_1-C_{10})alkyl, aryloxy(C_1-C_{10})alkyl, C_1-C_{10} alkoxycarbonyl, aryloxycarbonyl, aryloyloxy, substituted alkoxy, fluoroalkyl, nitro, cyano, cyano(C_1-C_{10})alkyl, C_1-C_{10} alkanamido, aryloylamido, arylaminosulfonyl, sulfonamido, amidino, amido, alkylamido, dialkylamido, amino, alkylamino, dialkylamino, carobyl, carbamido, carboxy, heterocyclic radical, nitroalkyl, and -$(CH_2)_m$-Z-(C_1-C_{10} alkyl), where m is 1 to 8 and z is oxygen or sulfur.

The term "lower alkyl" refers to straight chain or branched alkyl groups of 1-6 carbon atoms, such as methyl, ethyl, 1-methylethyl, propyl, butyl, isobutyl, sec-butyl, t-butyl, pentyl, isopentyl, hexyl and the like. In one preferred embodiment, the lower alkyl is a methyl group. In another preferred embodiment, the lower alkyl is a methylethyl group.

The term "aryl" refers to an aromatic group having at least one carbocyclic aromatic group, which may be unsubstituted or substituted by one or more inert substituents as defined hereinabove.

The term "heterocyclyl" or "heteroaryl" refers to a ring containing one or more heteroatoms, for example oxygen, nitrogen, sulfur and the like, with or without unsaturation or aromatic character, optionally substituted with one or more inert substituents as defined hereinabove. Non-limiting examples of heterocyclic substituents are imidazole, pyrazole, pyrazine, thiazole, thiazine, oxazole, furan, dihydrofuran, tetrahydrofuran, pyridine, dihydropyridine, tetrahydropyridine, isoxazole and the like. Multiple rings may be fused, as in quinoline or benzofuran, or unfused as in 4-phenylpyridine.

The heterocyclic moiety is a one or two ringed moiety containing one or more heteroatoms, preferably nitrogens, which may be isolated or fused, for example and without being limited to - imidazole, pyrazole, pyrazine, pyridine, dihydropyridine, tetrahydropyridine, isoxazole, quinoline, isoquinoline and the like.

A "haloalkyl" group refers to an alkyl group as defined above, which is substituted by one or more halogen atoms, e.g. by F, Cl, Br or I. A "hydroxyl" group refers to an OH group. An "alkenyl" group refers to a group having at least one carbon-to-carbon double bond. A halo group refers to F, Cl, Br or I. An "arylalkyl" group refers to an alkyl bound to an aryl, wherein alkyl and aryl are as defined above. An example of an arylalkyl group is a benzyl group.

As contemplated herein, the present invention further encompasses analogs, derivatives, isomers, pharmaceutically acceptable salts and hydrates of the compounds defined by the present invention.

The term "isomer" includes, but is not limited to, optical isomers and analogs, structural isomers and analogs, conformational isomers and analogs, and the like. In one embodiment, this invention encompasses of various optical isomers of the compounds of the present invention. It will be appreciated by those skilled in the art that the compounds of the present invention contain at least one chiral center. Accordingly, these compounds exist in, and be isolated in, optically-active or racemic forms. Some compounds may also exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically active, polymorphic, or stereroisomeric form, or mixtures thereof. In one embodiment, the compounds are the pure (R)-isomers. In another embodiment, the compounds are the pure (S)-isomers. In another embodiment, the compounds are a mixture of the (R) and the (S) isomers. In another embodiment, the compounds are a racemic mixture comprising an equal amount of the (R) and the (S) isomers. It is well known in the art how to prepare optically-active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

This invention further includes derivatives of the compounds. The term "derivatives" includes but is not limited to ether derivatives, acid derivatives, amide derivatives, ester derivatives and the like. In addition, this invention further includes hydrates of the compounds described herein. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate and the like.

The derivatives of the compounds of the present invention can also be in the form of prodrugs. Prodrugs are considered to be any covalently bonded carriers that release the active parent drug according to Formula I in vivo, when such prodrug is administered to a mammalian subject. Prodrugs of the compounds of Formula I are prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compounds. Prodrugs include compounds of Formula I wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Examples of prodrugs include, but are not limited to, acetate,

formate and benzoate derivatives of alcohol and amine functional groups in the compounds of Formula I, and the like.

According to one embodiment the compounds of the present invention inhibit the interaction of GAGs with GAG specific ECAMs.

According to another embodiment the compounds of the present invention inhibit the interactions of HS-GAGs with selectins, specifically L-selectin and P-selectin.

According to yet another embodiment the compounds according to the compositions of the present invention bind directly to GAGs, specifically HS-GAG.

According to yet another embodiment the present invention provides a method for inhibiting cell adhesion and cell migration in vitro comprising the step of exposing the cell to at least one compound according to formula I in an amount sufficient to inhibit GAG to GAG specific ECAM interactions.

The inhibitory effect of the compounds of the present invention can be evaluated by several methods in vitro. Co-pending Israel Patent Application No. 153762 describes a method for screening of compounds capable of inhibiting the binding of GAGs to ECAMs, using an in vitro assay for measuring GAG-ECAM binding, exemplified herein below for the binding of L selectin/IgG to immobilized heparin. Another approach for such assays is the use of immobilized L-selectin, or L-selectin fused to protein domains other than IgG. The amount of bound L-selectin is determined by an ELISA assay using a monoclonal antibody conjugated to horseradish peroxidase. Fig. 1 shows the saturation curve of the L-selectin/IgG binding to heparin. As shown in Fig. 2, soluble heparin inhibited L-selectin/IgG binding to immobilized heparin. A mAb directed against the carbohydrate-binding domain of L-selectin (Dregg-55) inhibited L-selectin/IgG binding to heparin (Fig. 3), providing a further confirmation of the specificity of binding. The Dregg-55 antibody was also shown to inhibit L-selectin-dependent adhesion in vitro, neutrophil accumulation in vitro and inflammation in vivo (Co M.S. et al, 1999 Immunotechnology 493, 253-266). Thus, the experiment with Dregg-55 antibody shows that the assay according to the present invention is useful for discovery of compounds inhibiting cell interaction and infiltration and having therapeutic potential.

According to yet another embodiment the compounds of the present invention directly bind to HS-GAGs. They can therefore be employed for the detection of GAGs in biological samples, and treatment or prevention of diseases and disorders

mediated by GAGs.

The biological activity of the compounds according to formula I of the present invention may be assayed in a variety of systems. For example, a compound can be immobilized on a solid surface and adhesion of cells expressing HS-GAGs can be measured. The test compounds can also be tested for the ability to competitively inhibit binding between HS-GAGs and other proteins binding to HS-GAGs such as other cell adhesion molecules, cytokines or viral proteins. Many assay formats employ radioactive or non-radioactive labeled assay components. The labeling systems can be in a variety of forms. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art.

According to a further embodiment the compounds of the present invention inhibit leukocyte and neutrophil infiltration *in vivo*.

The ability of compounds of the present invention to reduce leukocyte migration to sites of acute inflammation was evaluated in BALB/c mice using a thioglycolate-induced model of peritonitis. In this animal model, interactions of L- and P- selectin with HS-GAGs have been implicated in neutrophil infiltration (Nelson, R.M., 1993, 82(11), 3253-3258; Xie, X. et al., 2000, J. Biol. Chem., 275, 34818-34825).

Compounds according to formula I of the present invention were shown to efficiently inhibit leukocyte and neutrophil migration into the peritoneal cavity. The compounds were also shown to reduce lymphocyte migration, evaluated in mice using Delayed Type Hypersensitivity (Lange-Asschenfeldt B. et al., Blood 2002;99:538-545).

Compounds of the present invention having the desired biological activity may be modified as necessary to provide desired properties such as improved pharmacological properties (e.g., in vivo stability, bio-availability), or the ability to be detected in diagnostic applications. Stability can be assayed in a variety of ways such as by measuring the half-life of the proteins during incubation with peptidases or human plasma or serum. A number of such protein stability assays have been described (for example, Verhoef et al., Eur. J. Drug Metab. Pharmacokinet., 1990, 15(2):83-93).

For diagnostic purposes, a wide variety of labels may be linked to the compounds, which may provide, directly or indirectly, a detectable signal. Thus, the compounds of the present invention may be modified in a variety of ways for a variety of end purposes while still retaining biological activity. In addition, various reactive

sites may be introduced at the terminus for linking to particles, solid substrates, macromolecules, or the like.

Labeled compounds can be used in a variety of in vivo or in vitro applications. A wide variety of labels may be employed, such as radionuclides (e.g., gamma-emitting radioisotopes such as technetium-99 or indium-111), fluorescent agents (e.g., fluorescein), enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chemiluminescent compounds, bioluminescent compounds, and the like. Those of ordinary skill in the art will know of other suitable labels for binding to the complexes, or will be able to ascertain such using routine experimentation. The binding of these labels is achieved using standard techniques common to those of ordinary skill in the art.

For in vivo diagnostic imaging to identify for example, sites of inflammation, radioisotopes are typically used in accordance with well-known techniques. The radioisotopes may be bound to the peptide either directly or indirectly using intermediate functional groups. For instance, chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules have been used to bind proteins to metallic ion radioisotopes.

The invention includes pharmaceutically acceptable salts of the heterocyclic compounds of the present invention. Pharmaceutically acceptable salts can be prepared by treatment with inorganic bases, for example, sodium hydroxide or inorganic/organic acids such as hydrochloric acid, citric acids and the like.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine,

hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

It is to be understood that, as used herein, references to the compounds according to formula I of the present invention are meant to also include the pharmaceutically acceptable salts thereof.

Pharmaceutical Formulations

The pharmaceutical compositions of the present invention can be formulated for administration by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise as an active ingredient at least one compound according to formula I and derivatives thereof as described herein above, further comprising an excipient or a carrier. During the preparation of the pharmaceutical compositions according to the present invention the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active ingredient to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size

of less than 200 mesh. If the active ingredient is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methylcellulose. The formulations can additionally include lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxybenzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The active ingredient is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about

500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer, which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the compositions of the present invention may be incorporated, for administration orally or by injection, include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insulation include solutions and suspensions in pharmaceutically acceptable aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described above. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices that deliver the formulation in an appropriate manner.

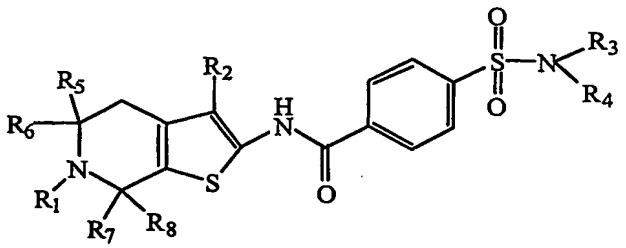
Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Pat. No. 5,023,252 incorporated herein by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Direct or indirect placement techniques may be used when it is desirable or necessary to introduce the pharmaceutical composition to the brain. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Pat. No. 5,011,472 incorporated herein by reference. Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions, which can transiently open the blood-brain barrier.

Therapeutic use

The present invention provides small organic compounds that inhibit cell-matrix and cell-cell interaction, thus inhibiting a cascade of events that lead to the development of certain diseases and disorders.

According to some aspects the present invention provides a method for the treatment or prevention of diseases and disorders related to cell adhesion and cell migration mediated by GAG-ECAM interactions, comprising the step of administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one molecule having the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with

alkyl groups; alkanoyl; arylcarbonyl optionally substituted at the aryl group; cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain C_1-C_6 alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of C_1-C_6 alkyl, alkoxy alkyl, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl, or R_3 and R_4 together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms;

R_5, R_6, R_7 and R_8 are selected from the group consisting of H or C_1-C_6 alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are C_1-C_6 alkyl, R_1 is hydrogen;

and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

According to one embodiment the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders related to GAG-ECAM interactions wherein the GAGs are selected from the group consisting of heparan sulfate (HS-GAG), heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate, and derivatives and fragments thereof.

According to one currently preferred embodiment, the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders related to GAG-ECAM interactions wherein the GAG is HS-GAG.

According to yet another embodiment the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders related to GAG-ECAM interactions wherein the GAG specific ECAMs are selected from the group consisting of selectins, integrins fibronectin and cytokines.

According to one currently preferred embodiment, the pharmaceutical compositions according to the present invention are used for the treatment of diseases or disorders related to GAG-ECAM interactions wherein the GAG specific ECAMs are selected from the group consisting of L-selectin and P-selectin.

Anti cell adhesion and anti-cell migration therapy has proven to be highly

effective in the treatment of number of diseases and disorders including inflammatory processes, autoimmune processes, cancer and platelet-mediated pathologies.

A number of inflammatory disorders are associated with L- and P- selectin or selectin-mediated leukocyte flow along the blood stream. Treatable disorders include, e.g., transplantation rejection (e.g., allograft rejection), atherosclerosis, retinitis, cancer metastases, rheumatoid arthritis, acute leukocyte-mediated lung injury (e.g., adult respiratory distress syndrome), asthma, allergic rhinitis, allergic conjunctivitis, inflammatory lung diseases, organ transplantation rejection, restenosis, autologous bone marrow transplantation, nephritis, and acute and chronic inflammation, atopic dermatitis, psoriasis, contact dermal hypersensitivity, myocardial ischemia, and inflammatory bowel disease. In preferred embodiments the pharmaceutical compositions are used to treat inflammatory disorders associated with neutrophil infiltration, such as ischemia-reperfusion injury, acute pancreatitis, septic shock, uveitis, rheumatoid arthritis and inflammatory bowel disease.

Reperfusion injury is a major problem in clinical cardiology. Therapeutic agents that reduce leukocyte adherence in ischemic myocardium can significantly enhance the therapeutic efficacy of thrombolytic agents. Thrombolytic therapy with agents such as tissue plasminogen activator or streptokinase can relieve coronary artery obstruction in many patients with severe myocardial ischemia prior to irreversible myocardial cell death. However, many such patients still suffer myocardial necrosis despite restoration of blood flow. This "reperfusion injury" is known to be associated with adherence of leukocytes to vascular endothelium in the ischemic zone, presumably in part because of activation of platelets and endothelium by thrombin and cytokines that makes them adhesive for leukocytes (Romson et al., Circulation 67:1016-1023, 1983). These adherent leukocytes can migrate through the endothelium and ischemic myocardium just as it is being rescued by restoration of blood flow.

Inflammatory bowel disease is a collective term for two similar diseases referred to as Crohn's disease and ulcerative colitis. Crohn's disease is an idiopathic, chronic ulceroconstrictive inflammatory disease characterized by sharply delimited and typically transmural involvement of all layers of the bowel wall by a granulomatous inflammatory reaction. Any segment of the gastrointestinal tract, from the mouth to the anus, may be involved, although the disease most commonly affects the terminal ileum and/or colon. Ulcerative colitis is an inflammatory response limited largely to the colonic mucosa and submucosa. Lymphocytes and macrophages are

numerous in lesions of inflammatory bowel disease and may contribute to inflammatory injury.

Asthma is a disease characterized by increased responsiveness of the tracheobronchial tree to various stimuli potentiating paroxysmal constriction of the bronchial airways. The stimuli cause release of various mediators of inflammation that recruit basophils, eosinophils and neutrophils, which cause inflammatory injury.

Rheumatoid arthritis is a chronic, relapsing inflammatory disease that primarily causes impairment and destruction of joints. Rheumatoid arthritis usually first affects the small joints of the hands and feet but then may involve the wrists, elbows, ankles and knees. The arthritis results from interaction of synovial cells with leukocytes that infiltrate from the circulation into the synovial lining of the joints.

Atherosclerosis is a disease of arteries. The basic lesion, the atheroma, consists of a raised focal plaque within the intima, having a core of lipid and a covering fibrous cap. Atheromas compromise arterial blood flow and weaken affected arteries. Myocardial and cerebral infarcts are a major consequence of this disease. Macrophages and leukocytes are recruited to atheromas and contribute to inflammatory injury.

The pharmaceutical compositions of the present invention can be further used in the treatment of organ or graft rejection. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immnunotolerance in the recipient to the transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host, the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue. $CD8^+$ cells, CD4 cells and monocytes are all involved in the rejection of transplant tissues. Compounds of this invention, which inhibit selectins are useful, inter alia, to block alloantigen-induced immune responses thereby preventing such cells from participating in the destruction of the transplanted tissue or organ (see, e.g., Georczynski et al., Immunology 87, 573-580 (1996); Yang et al., Transplantation 60, 71-76 (1995)).

A related use of the pharmaceutical compositions according to the present invention is in modulating the immune response involved in "graft versus host" disease (GVHD). GVHD is a potentially fatal disease that occurs when

immunologically competent cells are transferred to an allogeneic recipient. In this situation, the donor's immunocompetent cells may attack tissues in the recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the course of GVHD. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow transplantation; but less severe GVHD has also been reported in other cases as well, including heart and liver transplants. The compounds of the present invention that modulate donor cell homing pattern mediated by L-selectin, are useful for treatment of GVHD (Li, B., 2001, Eur J Immunol 2001 Feb;31(2):617-24).

Further use of the pharmaceutical compositions according to the present invention is for the treatment of metastasis. For certain cancers to spread throughout a patient's body, a process of cell-cell adhesion, or metatasis, must take place. Specifically, cancer cells must migrate from their site of origin and gain access to a blood vessel to facilitate colonization at distant sites. A critical aspect of this process is adhesion of cancer cells (to platelets and to endothelial cells that line the blood vessel wall) a step prior to migrating into surrounding tissue. This process can be interrupted by the administration of compounds of the invention, which generally aid in blocking cell-cell adhesion. In particular, P-selectin mediated processes have been implicated in metastasis (Varki, A. and Varki, N.M., Braz J Med Biol Res. 2001 Jun;34(6):711-7)

Also embodied is the use of the pharmaceutical compositions according to the present invention in the treatment of leukemia, which involves extravasation of leukemic cells and tumor formation, such as Acute Myeloid Leukemia. Also embodied in the present invention are methods useful for the treatment (including prevention) of angiogenic disorders. The term "angiogenic disorders" as used herein includes conditions involving abnormal neovascularization, such as tumor metastasis and ocular neovascularization, including, for example, diabetic retinopathy and neovascular glaucoma.

A further use of the pharmaceutical compositions according to the present invention is in treating multiple sclerosis. Multiple sclerosis is a progressive neurological autoimmune disease that is thought to be the result of a specific autoimmune reaction in which certain leukocytes initiate the destruction of myelin, the insulating sheath covering nerve fibers. Murine monoclonal antibodies directed against L-selectin have been shown to suppress experimental autoimmune

encephalomyelitis (EAE), an animal model of multiple sclerosis (Archelos, J.J., J. Neurol. Sci. 1998 Aug 14;159(2):127-34).

It has also been found that compounds according to formula I of the present invention directly bind to HS-GAGs and may therefore be useful for treatment of disease conditions mediated by HS-GAGs. HS-GAG mediated conditions include those mediated by cell-cell, cell-virus, cell-matrix and cell-protein interactions. Examples of HS-GAG mediated conditions include virus attachment to cell, cell adhesion, platelet aggregation, lymphocyte adhesion and migration, and amyloid fibril formation.

According to one embodiment, the pharmaceutical compositions according to the present invention are therefore also used for treatment (or prevention) of viral disorders such as hepatitis C and B, cytomegalovirus infection, respiratory syncytial virus infection and AIDS.

According to yet another embodiment, the pharmaceutical compositions of the present invention are used for the treatment or prevention of coagulation disorders, atherosclerosis, amyloid disorders including Alzheimer's disease and type II diabetes (Non-insulin Dependent Diabetes Mellitus), inflammatory and immune disorders, cancer, bone degradation, osteoporosis, osteoarthritis, tumor metastasis and kidney disease including glomerulonephritis.

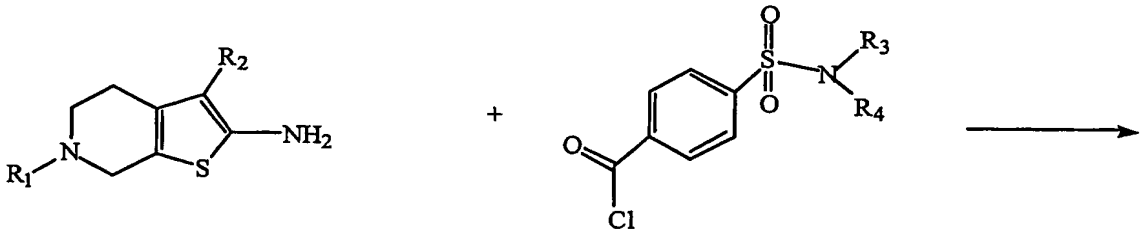
It is to be understood that while the compounds according to formula I of the present invention were selected for their capacity to inhibit binding of certain selectins to HS-GAGs, and that this property contributes to their medical activity, it cannot, however, be excluded that the compounds are also exerting their favorable medical effects, either in parallel or in tandem, through additional mechanisms of action. Thus, the skilled practitioner of this art will appreciate that one aspect of the present invention is the description of novel pharmaceutical compositions, and that Applicants intend not to be bound by a particular mechanism of action that may account for their prophylactic or therapeutic effects.

The principles of the invention, providing novel compounds capable of inhibiting GAGs-ECAMs interactions, their pharmaceutical compositions and use thereof according to the present invention, may be better understood with reference to the following non-limiting examples.

EXAMPLES

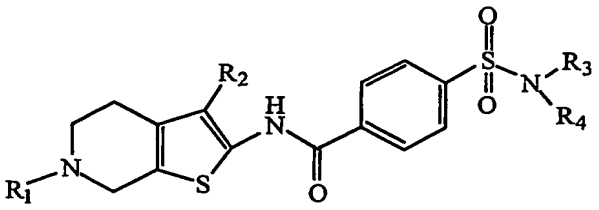
Example 1: General Synthesis of Compounds of Formula I

Compounds of Formula I are synthesized according to scheme of the reaction described by Noravyan et al. (A.S. Noravyan, A.P. Mkrtchyan, R.A.Akopyan and S.A.Vartanyan (1980), Khim.-Farm. Zh., 14(2), 37-40). A compound according to formula A herein below (R_1 and R_2 are defined as in formula I) is reacted with an acid chloride of the compound according to formula B herein bellow (where R_3 and R_4 are defined as in formula I) in dry benzene under reflux, in the presence of triethylamine. The precipitated crystals of triethylamine hydrochloride are filtered off and the filtrate is evaporated under slight vacuum. Work up of the residue affords the target compounds of formula I in 60-80 % yield.



A

B



I

Example 2: Pharmaceutical compositions

The pharmaceutical compositions of the present invention are illustrated by the following formulation examples:

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5.0
Lactose	95.0

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling-appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0
Starch	45.0
Microcrystalline cellulose	35.0
Polyvinylpyrrolidone (as 10% solution in water)	4.0
Sodium carboxymethyl starch	4.50
Magnesium stearate	0.5
Talc	1.0

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinyl-pyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50°C to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0
Starch	109.0
Magnesium stearate	1.0

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Quantity (mg)
Active Ingredient	25.0
Saturated fatty acid glycerides	2000.0

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 ml dose are made as follows:

Ingredient	Quantity (mg)
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%) Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v. mg
Purified water	to 5.0 ml

The medicament, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0
Starch	407.0
Magnesium stearate	3.0

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed

through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 560 mg quantities.

Formulation Example 9

An intravenous formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	250.0 mg
Isotonic saline	1000 ml

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

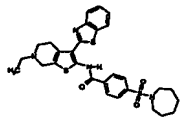
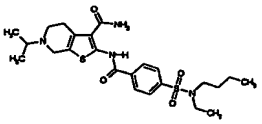
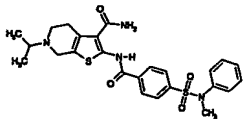
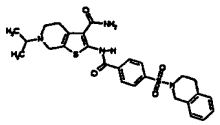
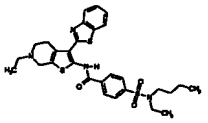
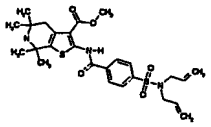
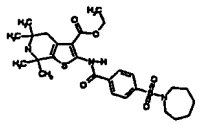
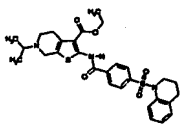
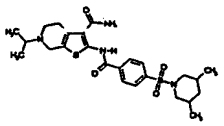
Example 3: In vitro assay for determining inhibition of L-selectin (P-selectin) binding to HS-GAGs by candidate compounds according to formula I

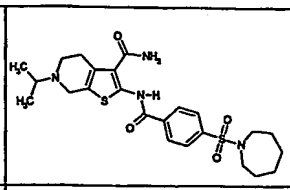
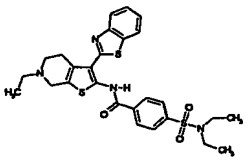
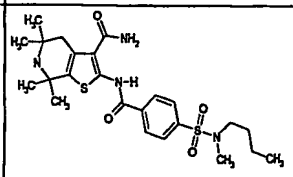
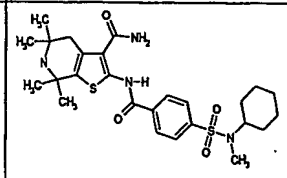
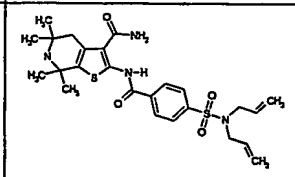
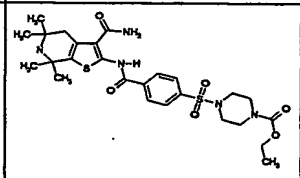
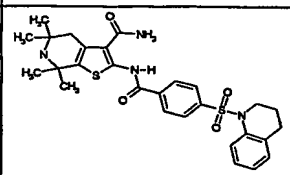
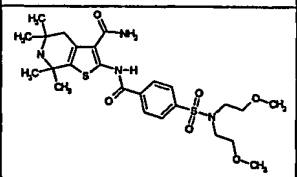
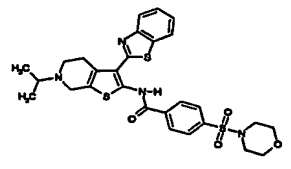
An in vitro assay was used to assess the ability of candidate compounds according to formula I to inhibit the interactions of L-selectin with HS-GAGs. This assay is suitable for determining the concentration required for 50% inhibition (IC-50) for each specific compound. In this assay, heparin was used instead of HS-GAG. Porcine intestinal mucosa heparin conjugated to Bovine Serum Albumin (Heparin-BSA; Sigma Cat.No. H0403) at 5mg/ml in Phosphate Buffered Saline (PBS; pH 6.5) was added to a 96 well polystyrene ELISA plate (NUNC Cat. No. 442404; 0.1ml per well) and incubated Over Night (ON) at 4°C. Following the incubation the plate was washed consecutively, by immersion, with de-ionized water and PBS (pH 6.5). The ELISA plate was then blocked with BSA (ICN Cat.No.160069, 3%, 200 µl per well)

for 1 hour at room temperature (RT). Following blocking, the plate was washed with de-ionized water then PBS (pH 6.5) plus Tween. Compounds were purchased from ChemDiv Labs (San Diego, CA), dissolved in DMSO, diluted in PBS and added to the wells at various concentrations in the range of 0.01 to 300 μM. Recombinant Human L-Selectin/IgG (Research and Development Systems Cat.No.728-LS) dissolved in PBS (supplemented with BSA (0.1%) and calcium chloride (1mM)) was added to the ELISA plate (100 μl per well) and incubated for 60 minutes at RT with shaking. Following incubation, the plate was washed with de-ionized water and three times with PBS (pH 6.5) plus Tween. The peroxidase substrate cromogen, TMB (Dako Cat. No. S1599) was added (100 ul per well) to the ELISA plate and incubated at room temperature. After 15 minutes ELISA Stop Solution (hydrochloric acid 1N, sulfuric acid 3N) was added (200 μl per well) to stop the peroxidase catalyzed colorimetric reaction. The Optical Density of the samples was measured at 450nm using an ELISA plate reader (Dynatech MR5000). Data were analyzed with Graphpad Prism software and IC-50 values were established. The P-selectin assay was carried out in a similar fashion, except that Recombinant Human P-Selectin/IgG (Research and Development Systems Cat.No.137-PS) was used.

It was established that compounds of Formula I had inhibitory activity in the above assays in the micromolar range. Examples of Inhibitor Compounds are given in Table 1.

Table I: Inhibition of L-Selectin/IgG Binding to Heparin by Selected Compounds

Compound No.	Structure	% Inhibition at 30 μM	IC-50 [μM]
1		25	
2		31	
3		73	35
4		74	32.1
5		49	13
6		67	56.4
7		50	
8		32	
9		35	

10		27	
11		70	5
12		49	
13		65	
14		36	
15		33	
16		71	
17		32	
18		48	7.4

Example 4: An assay to demonstrate direct interaction of Inhibitor Compounds with heparin and other HS-GAGs.

In order to demonstrate that the L-selectin Hit Compounds indeed bind directly to heparin and other HS-GAGs, individual compounds were incubated with immobilized heparin in the absence of L-selectin/IgG. 96 well ELISA plates were coated with Heparin-BSA, then blocked with BSA as described in Example 1. L-selectin Hit Compounds, at final concentration 0.1-200 μM, were incubated in the ELISA plate for 90 min, and then washed with incubation buffer. After washing, L-selectin/IgG was added to the wells pre-incubated with compounds. At the same time, in separate control wells, L-selectin was co-incubated with L-selectin Hit Compounds for 90min. Following the incubation, L-selectin bound to the plate was quantified by antibody conjugated to Horse Radish Peroxidase and OD measurement as described in Example 3. L-selectin/heparin Inhibitor Compounds No. 5 and No. 11 inhibited L-selectin binding to heparin to the same extent in pre-incubation vs. co-incubation experiments.

Example 5: A model of leukocyte and neutrophil infiltration into mouse peritoneum

BALB/c mice were 6 weeks old, about 20 g in weight. The animals (15 mice/group) received intraperitoneal injection of test compound in 0.2 ml DMSO/Tween/sterile saline 1 hour before administration of thioglycollate (Sigma). Control groups received vehicle and sham controls received no thioglycollate. Mice were injected intraperitoneally with 1 ml of 3% thioglycollate broth (Xie, X. et al.: J. Biol. Chem., 275, 44, 34818–34825, 2000). Mice were sacrificed after 3 hours, and the peritoneal cavities were lavaged with 5 ml of ice-cold saline containing 2 mM EDTA to prevent clotting. After red blood cell lysis, leukocytes were counted in a hemocytometer. Neutrophils were counted after staining with Türck. Data was expressed as mean ± SEM, and statistical analysis was performed by Student *t* test. A value of $P<0.05$ was taken to denote statistical significance.

Thioglycollate administration induced approximately 3-fold increase in leukocyte accumulation in the peritoneal cavity. Leukocyte migration into the peritoneal cavity was efficiently inhibited by administration of test compounds including compound No. 5 and 11.. Similar results were obtained when the neutrophil

counts were determined. Compound no. 5 was tested in more detail at three doses, 2 mg/kg, 10 mg/kg and 50 mg/kg (Fig. 4). The compound is a potent inhibitor of leukocyte migration; the infiltration was reduced by 75% at a dose of 50 mg/kg, by 50% at 10 mg/kg and by 25% at 2 mg/kg. Leukocyte migration and infiltration in vivo is a hallmark of inflammatory, autoimmune and other disorders. The ability of these compounds to inhibit leukocyte infiltration in vivo has therefore therapeutic applications for these disorders.

Example 6: Carrageenan-induced paw edema

Acute edema was induced in the left hind paw of Balb/c mice by injecting 0.02 ml of freshly prepared solution of 2% carrageenan after 60 min of test drugs administration (Carrageenan-induced paw edema: Torres, S.R. et al., European Journal of Pharmacology 408 2000 199–211). The right paw received 0.02 ml of saline, which served as control. Carrageenan was injected under the plantar region of right hind paw and the paw thickness was measured at 2, 4 and 24 hours after carrageenan challenge using a Mitutoyo engineer's micrometer expressed as the difference between right and left pad as mean ± SEM. Test compounds significantly reduced carrageenan induced paw edema after i.p. administration. A dose response curve for compound no. 5 is shown in Fig. 5. These results demonstrate that compounds inhibiting GAG binding to GAG-ECAMs display anti-inflammatory activity.

Example 7: Delayed-type hypersensitivity (DTH)

Mice (15 animals per group) were sensitized by topical application of a 2% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazoline- 5-one; Sigma, St Louis, MO) solution in acetone/olive oil (4:1 vol/vol) to shaved abdomen (50 µl) and to each paw (5 µl) (Lange-Asschenfeldt B. et al., Blood 2002;99:538-545). Five days after sensitization, right ears were challenged by topical application of 10 µl of a 1% oxazolone solution, whereas left ears were treated with vehicle alone. The extent of inflammation was measured 24 hours after challenge, using the mouse ear-swelling test. The unpaired Student *t* test was used for statistical analyses.

As illustrated in Fig. 6, compound no. 5 (at a dose of 3 mg/kg, administered iv) inhibited DTH to 56% of control value 24 hours after challenge. Data were

statistically significant at p>0.001.

Example 8: A mouse model of kidney ischemia/reperfusion

Male Balb/c mice weighing 20 g from Velaz (Prague, Czech Republic) are housed individually in standard cages with access to food and water ad libitum. (These kinds of studies are approved by the Institutional Animal Care Committee). 30 minutes of unilateral ischemia of the left kidney is followed by contralateral nephrectomy, as described in detail previously (Daemen, M. et al., J. Clin. Invest. 104:541–549, 1999). The animals are euthanized at defined time points. At the time of euthanization, blood is collected by orbital puncture, and the left kidney is harvested. Renal neutrophil accumulation is quantified by measuring renal myeloperoxidase content as described. Myeloperoxidase activity is expressed per milligram tissue by comparing the optical density of samples with a horseradish peroxidase titration curve and standardized with respect to wet/dry ratios. Blood urea nitrogen (BUN) content and serum creatinine levels are measured in serum by using a BUN Unimate 5 kit and a CREA MPR3 kit (Boehringer-Mannheim) in a Cobas Fara autoanalyzer (Roche). Kidney specimens are immediately frozen and stored in liquid nitrogen or fixed in buffered formalin and embedded in paraffin. Frozen sections (5 mm) are stained for neutrophils with mAb Gr-1 as described. Data are expressed as mean ± SEM, and statistical analysis is performed by Student *t* test. A value of $P < 0.05$ is taken to denote statistical significance.

Example 9: Cecal Ligation and Puncture (CLP)

An animal model for septic shock is described according to Godshall, C.J. et al. (Journal of Surgical Research 102, 45–49, 2002). Male BALB/c mice (20 g) are anesthetized with ketamine (87 mg/g) (Ketaset; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and xylazine (13 mg/g) (Rompun; Bayer Corporation, Shawnee Mission, Kans.), and a 2-cm midline incision is made through the linea alba. The cecum is located, ligated with sterile 3-0 silk, and perforated with18-gauge needle. A small amount of stool is extruded to ensure wound potency. Sham-treated mice also have surgery done along with cecal manipulations but without ligation and puncture. The cecum is then replaced in its original position within the abdomen, which is

closed in two layers. Immediately after surgery, each mouse received a subcutaneous injection of 1 ml of warm normal saline (37°C) and is placed in an incubator (37°C) for 15 min. The mice are then moved to a closed room and maintained at 22°C for the remainder of the experiment. Mice are killed at 4 h after CLP and lung tissue is collected for determination of myeloperoxidase levels.

Example 10: Dextran sulfate induced colitis

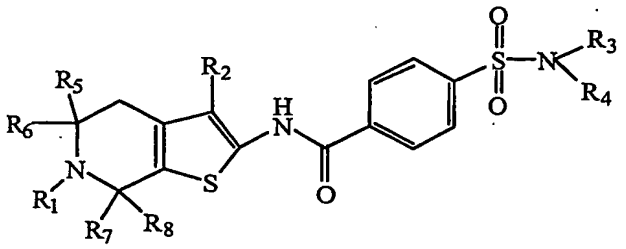
Colonic inflammation is induced by the administration of dextran sulfate (DSS) in the drinking water as described (Kato S. et al., J. Pharmacol. Exp. Therapeutics 2000, 295, 183-189). The animals are exposed to 5% DSS ad libitum. Mice are treated via one single oral gavage with compound at 10 mg/kg twice a day (5 mg/kg dose) or vehicle starting on day 1 and continuing through the study. The following parameters are recorded: mortality, body weight, colon length, colon histology and myeloperoxidase levels.

Example 11: Acute Pancreatitis

Animals (15 per group) are treated hourly (5 times) with cerulein (50 microg/kg, suspended in saline solution, i.p.) to induce acute pancreatitis (Cuzzocrea, S. et al., 2002, Cytokine 18, 274-285). Mice are killed by cervical dislocation 6 h after the administration of cerulein. Blood samples are obtained by direct intracardiac puncture. Pancreases are immediately removed, frozen in liquid nitrogen, and stored at 80 C until required for biochemical assay. Pieces of organ are also fixed in formaldehyde for histology or immunohistochemistry. After cerulein or saline administration, animals are monitored for evaluation of mortality for 5 days. Acinar-cell injury/necrosis is quantified by morphometry. Serum amylase and lipase levels are measured at 6 h after cerulein injection as described. Myeloperoxidase (MPO) activity is measured to assess neutrophil infiltration. Briefly, after weighing, sections of the pancreas are suspended in 0.5% hexadecyltrimethylammonium bromide (pH 6.5, 50 mg of tissue per ml) and is then homogenized. After freezing and thawing the homogenate three times, the tissue levels of MPO are determined by utilizing 0.0005% hydrogen peroxide as a substrate for the enzyme.

CLAIMS

1. A pharmaceutical composition comprising as an active ingredient a compound of the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with alkyl groups; alkanoyl; arylcarbonyl optionally substituted at the aryl group; cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain C_1-C_6 alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of C_1-C_6 alkyl, alkoxy alkyl, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl, or R_3 and R_4 together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms;

R_5, R_6, R_7 and R_8 are selected from the group consisting of H or C_1-C_6 alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are C_1-C_6 alkyl, R_1

is hydrogen;

and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

2. The pharmaceutical composition according to claim 1, wherein R_1 is selected from the group consisting of methyl, ethyl, 1-methylethyl, phenylmethyl, acetyl, ethoxycarbonyl and R_5 =R_6 =R_7 =R_8 are hydrogens.

3. The pharmaceutical composition according to claim 1, wherein R_1 is hydrogen and R_5 =R_6 =R_7 =R_8 are hydrogens or methyl groups.

4. The pharmaceutical composition according to claim 1, wherein R_1 =R_5 =R_6 is methyl and R_7 =R_8 are hydrogens.

5. The pharmaceutical composition according to claim 1, wherein R_2 is selected from the group consisting of cyano, methoxycarbonyl, ethoxycarbonyl, aminocarbonyl, methylaminocarbonyl, dimethylaminocarbonyl, pyrrolidinylcarbonyl, piperidinylcarbonyl, morpholinylcarbonyl, benzothiazol-2-yl.

6. The pharmaceutical composition according to claim 1, wherein R_3 and R_4 are selected from the group consisting of methyl, ethyl, propyl, butyl, methoxyethyl, chlorobutyl, cyanoethyl, phenyl, cyclopentyl, cyclohexyl, phenylmethyl, allyl or crotyl, R_3 and R_4 may be equal or different.

7. The pharmaceutical composition according to claim1, wherein R_3 and R_4 form pyrrolidine, piperidine, 2-methyl, 3-methyl, 4-methyl or 3,5-dimethyl piperidine, perhydroazepine, morpholine, piperazine, 3,4-dihydro-1(2H)quinoline, 1,3,3-trimethyl-6-azabicyclo[3.2.1]oct-6-ane and their substituted derivatives such as piperazinyl-4-caboxylic acid ester, piperidinyl-4-carboxylic acid ester, piperidinyl-3-carboxylic acid ester.

8. The pharmaceutical composition according to claim 1 wherein the compound of Formula I is selected from:

2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[(4-(3,4-dithydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxamide ;

2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(morpholinylsulfonyl) benzoyl]amino]-3-(benzothiazol-2-yl)-6-(1-methylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine ;

2-[[4-(diethylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester ;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine ;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl thieno[2,3-c]pyridine ;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo [3.2.1]oct-6-yl)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(methylphenylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-(morpholinylsulfonyl) benzoyl] amino]-3-(benzothiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine.

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-6-carboxylic acid ethyl ester;

2-[[4-[[4-(3-methyl-1-piperidinyl)]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-(phenylmethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[(diethylamino)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine;

2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-

4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(cyclohxylmethylamino}sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(di-2-propenylamino)sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxylic acid methyl ester;

2-[[4-[(di-2-methoxyethylamino)]sulfonyl]benzoyl]-4,5,6,7-tetrahydro-5,5,7,7-tetramethylthieno[2,3-c]pyridine-3-carboxamide;

2-[[4-[(1,3,3-trimethyl-6-azabicyclo[3.2.1.]okt-6-yl)sulfonyl]benzoyl]amino] –6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide.

9. The pharmaceutical composition according to claim 8 wherein the compound of formula I is:
4,5,6,7-tetrahydro-2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl thieno[2,3-c]pyridine.

10. The pharmaceutical composition according to claim 8 wherein the compound of formula I is:
4,5,6,7-tetrahydro-2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl- thieno[2,3-c]pyridine.

11. The pharmaceutical composition according to any one of claims 1-10 capable of inhibiting the interaction of GAGs with GAG specific ECAMs.

12. The pharmaceutical composition according to claim 11 wherein the GAG is selected from the group consisting of heparan sulfate (HS-GAG), heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate and derivatives and fragments thereof.

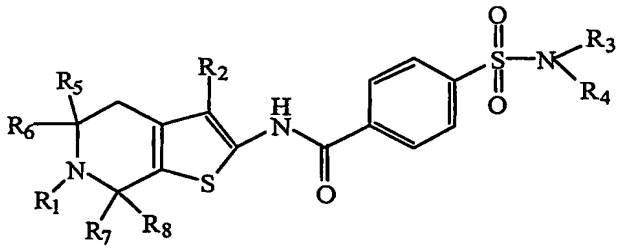
13. The pharmaceutical composition according to claim 12 wherein the GAG is HS-GAG.

14. The pharmaceutical composition according to claim 11 wherein the GAG specific ECAMs are selected from the group consisting of L-selectin and P-selectin.

15. A method for inhibiting cell adhesion or cell migration in vitro comprising the step of exposing the cells to a pharmaceutical

composition according to any one of claims 1-14 in an amount sufficient for preventing the interactions of the GAG with at least one GAG specific ECAM.

16. A method for the treatment or prevention of diseases or disorders related to cell adhesion or cell migration mediated by GAG-ECAM interactions, comprising the step of administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a compound of the general formula I:



wherein:

R_1 is selected from the group consisting of H; straight or branched alkyl of 1-6 carbon atoms; arylalkyl; substituted arylalkyl; cycloalkyl, optionally substituted with lower alkyl groups; lower alkanoyl; arylcarbonyl optionally substituted at the aryl group; cycloalkylcarbonyl; alkoxycarbonyl;

R_2 is selected from the group consisting of carboxy; cyano; aminocarbonyl; alkylaminocarbonyl; arylaminocarbonyl optionally substituted at the aryl group; dialkylaminocarbonyl wherein each alkyl is straight or branched chain lower alkyl or both alkyl groups together may form a 3-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; alkoxycarbonyl; lower alkanoyl; cycloalkylcarbonyl; arylcarbonyl optionally substituted on the aryl group, benzothiazol-2-yl;

R_3 and R_4 are selected from the group consisting of lower alkyl, optionally substituted with one or two groups selected independently from R_9, C_2 –C_4 monounsaturated alkenyl, cycloalkyl, aryl, arylmethyl,

or R_3 and R_4 may together may form a 5-7 membered saturated monocyclic or bicyclic cycloalkyl or heterocyclyl containing one or two heteroatoms; R_5, R_6, R_7 and R_8 are selected from the group consisting of H or lower alkyl, with the proviso that when R_5, R_6, R_7 and R_8 are lower alkyl, R_1 is hydrogen;

R_9 is selected from the group consisting a halogen, alkoxy and cyano; and pharmaceutically acceptable salts thereof; further comprising a pharmaceutically acceptable diluent or carrier.

17. The method according to claim 16 wherein R_1 is selected from the group consisting of methyl, ethyl, 1-methylethyl, phenylmethyl, acetyl, ethoxycarbonyl and R_5 =R_6 =R_7 =R_8 are hydrogens.

18. The method according to claim 16 wherein R_1 is hydrogen and R_5 =R_6 =R_7 =R_8 are hydrogens or methyl groups.

19. The method according to claim 16 wherein R_1 =R_5 =R_6 is methyl and R_7 =R_8 are hydrogens.

20. The method according to claim 16 wherein R_2 is selected from the group consisting of cyano, methoxycarbonyl, ethoxycarbonyl, aminocarbonyl, methylaminocarbonyl, dimethylaminocarbonyl, pyrrolidinylcarbonyl, piperidinylcarbonyl, morpholinylcarbonyl, (3,5-dimethyl-1H-pyrazolyl) carbonyl, benzothiazol-2-yl.

21. The method according to claim 16 wherein R_3 and R_4 are selected from the group consisting of methyl, ethyl, propyl, butyl, methoxyethyl, chlorobutyl, cyanoethyl, phenyl, cyclopentyl, cyclohexyl, phenylmethyl, allyl or crotyl, R_3 and R_4 may be equal or different.

22. The method according to claim 16 wherein R_3 and R_4 form pyrrolidine, piperidine, 2-methyl, 3-methyl, 4-methyl or 3,5-dimethyl piperidine, perhydroazepine, morpholine, piperazine, 3,4-dihydro-1(2H)quinoline, 1,3,3-trimethyl-6-azabicyclo[3.2.1]oct-6-ane and their substituted derivatives such as piperazinyl-4-caboxylic acid ester, piperidinyl-4-carboxylic acid ester, piperidinyl-3-carboxylic acid ester.

23. The method according to claim 16 wherein the compound of formula I is selected from:

4,5,6,7-tetrahydro-2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[(4-(3,4-dithydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-6-(1-methylethyl)- thieno[2,3-c]pyridine-3-carboxamide;

4,5,6,7-tetrahydro-2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]-6-(1-methylethyl)-ester;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl]benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl- thieno[2,3-c]pyridine-3-carboxamide;

4,5,6,7-tetrahydro-2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl- thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[4-(morpholinylsulfonyl) benzoyl]amino]-3-(benzothiazol-2-yl)-6-(1-methylethyl)- thieno[2,3-c]pyridine;

2-[[4-(diethylamino)sulfonyl] benzoyl]amino]-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl- thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester;

2-[[4-(3,4-dihydro-1(2H)-quinolinyl)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-4,5,6,7-tetrahydro-5,5,7,7-tetramethyl- thieno[2,3-c]pyridine;

2-[[4-(methylphenylamino)sulfonyl] benzoyl]amino]4,5,6,7-tetrahydro-5,5,7,7-tetramethyl- thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[4-[[4-(ethoxycarbonyl)-1-piperazinyl]sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[4-[(1,3,3-trimethyl-6-azabicyclo [3.2.1]oct-6-yl)sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl-thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[4-[(methylphenylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-methyl- thieno[2,3-c]pyridine;

4,5,6,7-tetrahydro-2-[[4-(morpholinylsulfonyl) benzoyl] amino]-3-(benzothiazol-2-yl)-6-methyl- thieno[2,3-c]pyridine.

24. The method according to claim 23 wherein the compound of formula I is:

4,5,6,7-tetrahydro-2-[[4-[(ethylbutylamino) sulfonyl]benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl thieno[2,3-c]pyridine.

25. The method according to claim 23 wherein the compound of formula I

is:

4,5,6,7-tetrahydro-2-[[4-[(diethylamino)sulfonyl] benzoyl]amino]-3-(benzothiazol-2-yl)-6-ethyl- thieno[2,3-c]pyridine.

26. The method according to claim 16 wherein the disease or disorder related to cell adhesion or cell migration is selected from the group consisting of an inflammatory process, an autoimmune process or disease, an unwanted leukocyte or neutrophil infiltration, platelet-mediated pathologies, tumor metastasis, viral diseases, coagulation disorders, atherosclerosis, amyloid disorders, and kidney disease.

27. The method according to claim 26 wherein the inflammatory disorder is selected from the group consisting of septic shock, post-ischemic leukocyte-mediated tissue damage, frost-bite injury or shock, acute leukocyte-mediated lung injury, acute pancreatitis, asthma, traumatic shock, stroke, traumatic brain injury, nephritis, acute and chronic inflammation, including atopic dermatitis, psoriasis, uveitis, retinitis, and inflammatory bowel disease.

28. The method according to claim 26 wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis and multiple sclerosis.

29. The method according to claim 26 wherein the diseases and disorders associated with unwanted leukocyte or neutrophil infiltration are selected from the group consisting of inflammatory processes: post-ischemic leukocyte-mediated tissue damage, acute pancreatitis, septic shock, stroke, acute and chronic inflammation; Crohn's disease; rheumatoid arthritis; multiple sclerosis and leukemia.

30. A method of treatment or prevention of GAG mediated diseases or disorders comprising the step of administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a pharmaceutical composition according to anyone of claims 1-14.

31. The method according to claim 30 wherein the GAG is HS-GAG.

32. The method according to claim 30 wherein the disease or disorder is selected from the group consisting of amyloid disorder: Alzheimer's disease and type II diabetes; viral diseases: hepatitis C and B,

influenza, rhinovirus infections, cytomegalovirus infections, AIDS, respiratory syncytial virus infections, bacterial infections and malaria; kidney diseases; cancer and coagulation disorder.

For the applicants:

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Webb & Associates
Patent Attorneys

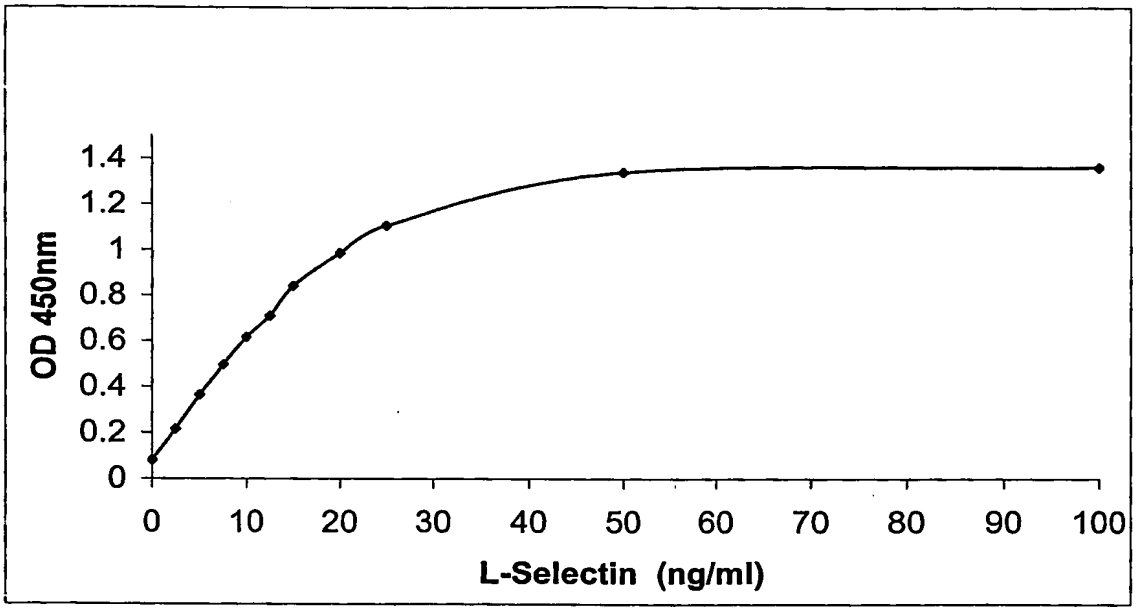
OD 450nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
90
100
L-Selectin (ng/ml)

FIGURE 1

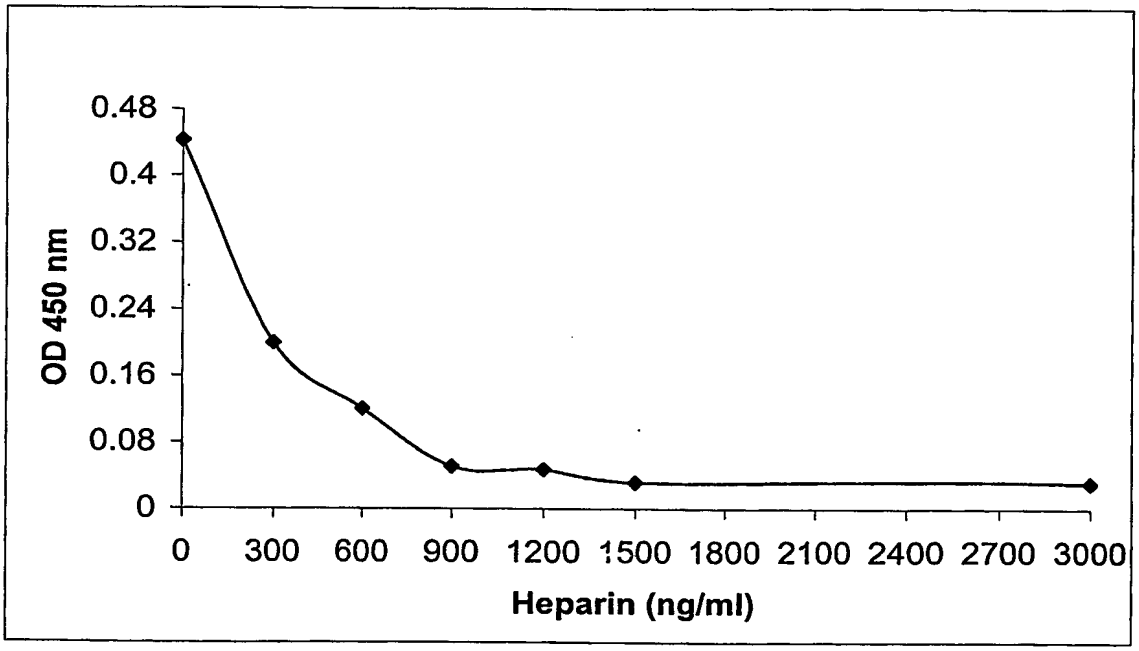
0.48
0.4
0.32
0.24
0.16
0.08
0
OD 450 nm
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
Heparin (ng/ml)

FIGURE 2

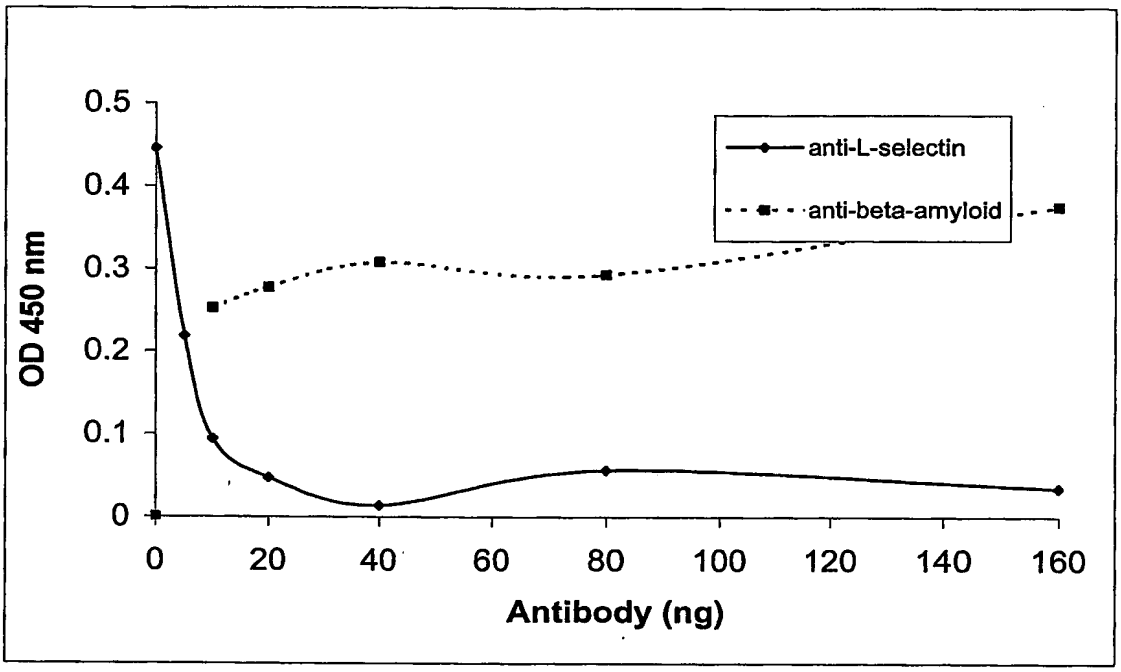
0.5
0.4
0.3
0.2
0.1
0
OD 450 nm
0
20
40
60
80
100
120
140
160
Antibody (ng)
anti-L-selectin
anti-beta-amyloid

FIGURE 3

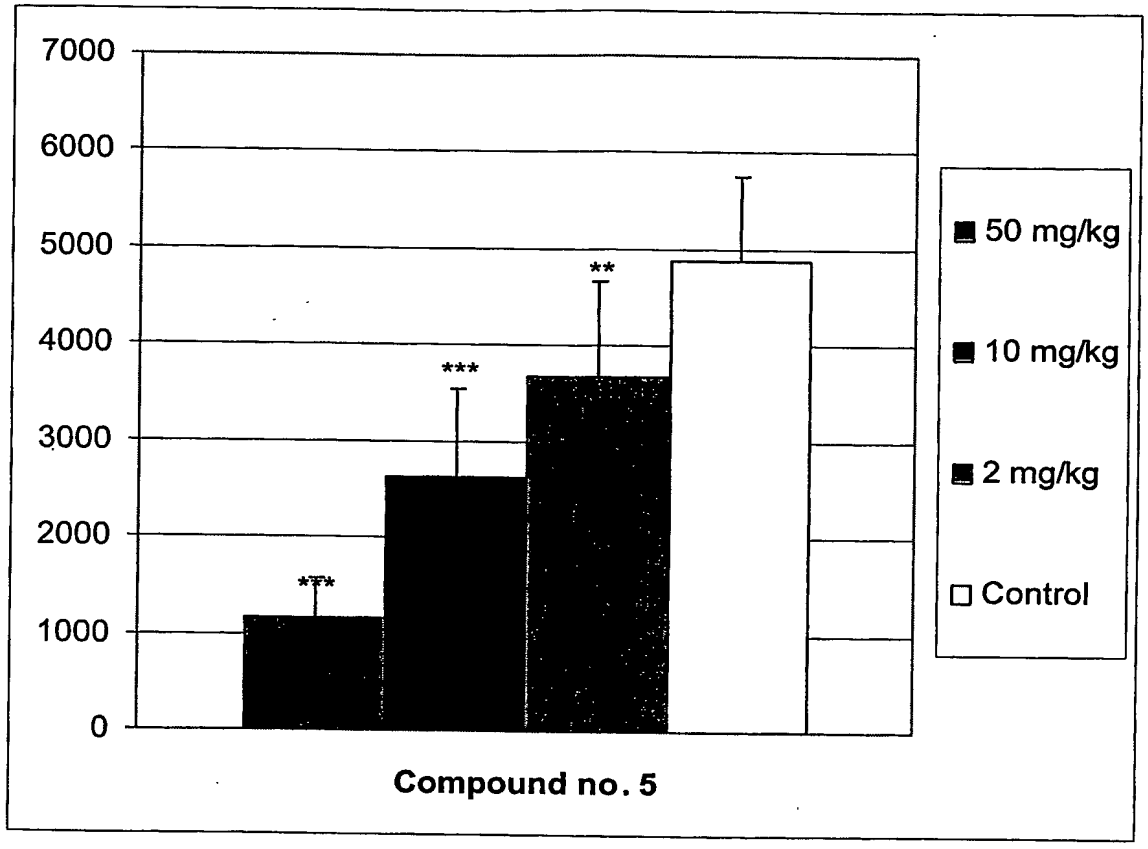
7000
6000
5000
4000
3000
2000
1000
0
***
**
***
50 mg/kg
10 mg/kg
2 mg/kg
Control
Compound no. 5

FIGURE 4

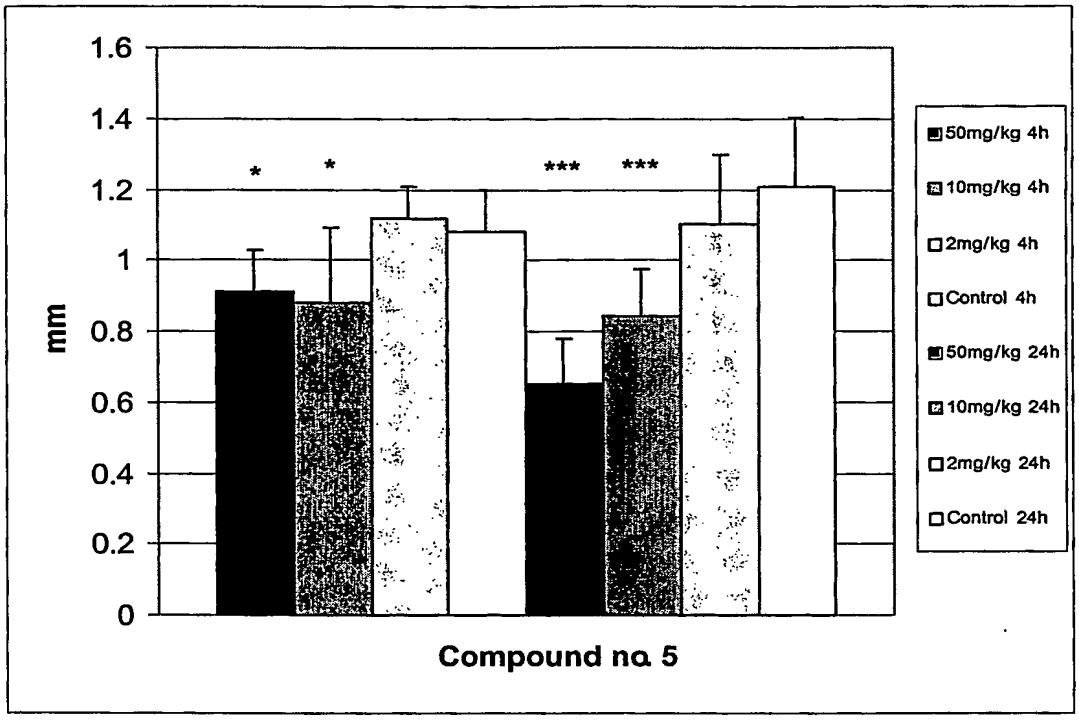
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
mm
*
*
***
***
Compound no. 5
50mg/kg 4h
10mg/kg 4h
2mg/kg 4h
Control 4h
50mg/kg 24h
10mg/kg 24h
2mg/kg 24h
Control 24h

FIGURE 5

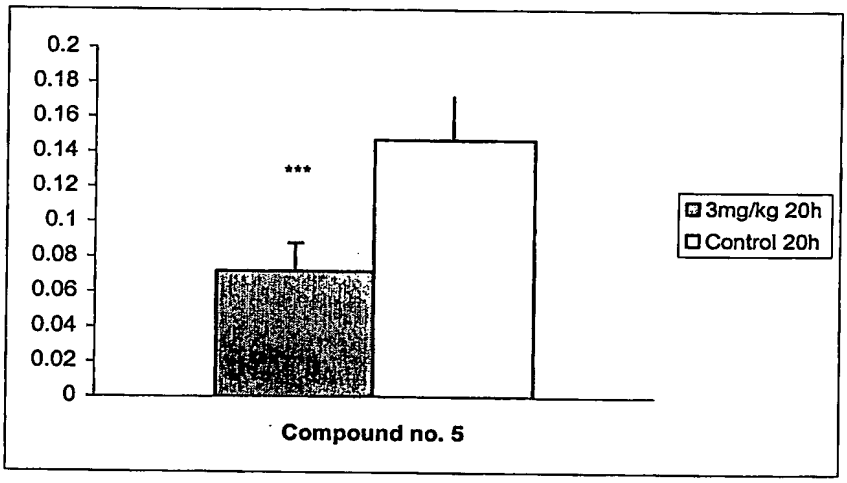
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
***
3mg/kg 20h
Control 20h
Compound no. 5

FIGURE 6